

Comparative Study of Xenobiotic-Free Media for the Cultivation of Human Limbal Epithelial Stem/Progenitor Cells.

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Public Summary:

The culture of human limbal epithelial stem/progenitor cells (LSCs) in the presence of animal components poses the risk of cross-species contamination in clinical applications. We quantitatively compared different xenobiotic-free culture media for the cultivation of human LSCs. LSCs were cultured from 2 x 2 mm limbal tissue explants on denuded human amniotic membrane with different xenobiotic-free culture media: CnT-Prime (CnT-PR) supplemented with 0%, 1%, 5%, and 10% human serum (HS), embryonic stem cell medium (ESCM) alone or in combination with the standard supplemented hormonal epithelium medium (SHEM, control) at a 1:1 dilution ratio, and modified SHEM (mSHEM), in which cholera toxin and dimethyl sulfoxide (DMSO) were removed, isoproterenol was added, and the epidermal growth factor concentration was reduced. Several parameters were quantified to assess the LSC phenotype: cell morphology, cell growth, cell size, outgrowth size, and expression of the undifferentiated LSC markers cytokeratin (K) 14, and p63alpha high-expressing (p63alphabright) cells, a mature keratinocyte marker K12, epithelial marker pancytokeratin (PanK), and stromal cell marker vimentin (Vim). Compared with the standard SHEM control, CnT-PR base medium was associated with a lower cell growth and reduction in the proportion of stem cells generated regardless of the amount of HS supplemented ($p < 0.05$); ESCM resulted in an increased proportion of PanK-/Vim+ stromal cells ($p < 0.05$) and a decreased proportion of p63alphabright cells ($p < 0.05$); mSHEM supported a similar cell growth ($p > 0.05$), increased the number of small cells (diameter $\leq 12 \mu\text{m}$; $p < 0.05$), and provided a similar proportion of p63alphabright cells ($p > 0.05$). Among all the conditions tested, mSHEM was the most efficient and consistent in supporting the LSC phenotype and growth.

Scientific Abstract:

The culture of human limbal epithelial stem/progenitor cells (LSCs) in the presence of animal components poses the risk of cross-species contamination in clinical applications. We quantitatively compared different xenobiotic-free culture media for the cultivation of human LSCs. LSCs were cultured from 2 x 2 mm limbal tissue explants on denuded human amniotic membrane with different xenobiotic-free culture media: CnT-Prime (CnT-PR) supplemented with 0%, 1%, 5%, and 10% human serum (HS), embryonic stem cell medium (ESCM) alone or in combination with the standard supplemented hormonal epithelium medium (SHEM, control) at a 1:1 dilution ratio, and modified SHEM (mSHEM), in which cholera toxin and dimethyl sulfoxide (DMSO) were removed, isoproterenol was added, and the epidermal growth factor concentration was reduced. Several parameters were quantified to assess the LSC phenotype: cell morphology, cell growth, cell size, outgrowth size, and expression of the undifferentiated LSC markers cytokeratin (K) 14, and p63alpha high-expressing (p63alphabright) cells, a mature keratinocyte marker K12, epithelial marker pancytokeratin (PanK), and stromal cell marker vimentin (Vim). Compared with the standard SHEM control, CnT-PR base medium was associated with a lower cell growth and reduction in the proportion of stem cells generated regardless of the amount of HS supplemented ($p < 0.05$); ESCM resulted in an increased proportion of PanK-/Vim+ stromal cells ($p < 0.05$) and a decreased proportion of p63alphabright cells ($p < 0.05$); mSHEM supported a similar cell growth ($p > 0.05$), increased the number of small cells (diameter $\leq 12 \mu\text{m}$; $p < 0.05$), and provided a similar proportion of p63alphabright cells ($p > 0.05$). Among all the conditions tested, mSHEM was the most efficient and consistent in supporting the LSC phenotype and growth.